Effects of NO₂, SO₂ and O₃ Alone and in Combinations on Net Photosynthesis in Sunflower

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The effects of NO₂, SO₂ and O₃ singly and in combinations on the rates of net photosynthesis were examined for intact leaves of sunflower (*Helianthus annuus* L. cv. Russian Mammoth). Concentrations of air pollutants used were 1.0 or 0.2 ppm for NO₂ and 0.2 ppm for both SO₂ and O₃.

Exposures to 1.0 ppm NO₂, 0.2 ppm SO₂, or 0.2 ppm O₃ separately caused no detectable depression of photosynthetic rates, but combinations of these two or three gases measurably reduced the rate to a greater degree than would be expected from the sum of the inhibitions caused by individual gases. NO₂ concentration in NO₂/SO₂ mixture was the variable that determined the degree of photosynthesis inhibition, but the magnitude of the photosynthesis depression caused by NO₂/O₃ or NO₂/SO₂/O₃ mixture was not influenced by the concentrations of NO₂ in the mixture.

The rate of photosynthesis inhibition caused by NO₂/O₃ mixture was quite different from that caused by SO₂/O₃ or NO₂/SO₂/O₃ mixture. The mixture of NO₂/O₃ decreased the rate of net photosynthesis steadily with duration of exposure, while the mixture of SO₂/O₃ or NO₂/SO₂/O₃ caused the rate to depress rapidly to the new equilibrium inhibition level.

Extensive research into the effects of air pollutants on vegetation has been conducted during the past century and a number of available reviews have been documented.^{4,6,9)} However, most studies have focussed attention on plant responses to a single air pollutants. Under the field conditions, pollutants rarely exist alone, but a complex mixture of phytotoxic gases affects plant. To better understanding of the effects of air pollutants on vegetation, it is necessary to define the response of plants to pollutant mixtures.

Recently, some workers have challenged to define the effects of pollutant mixtures on plant. There is a considerable information available in the literature concerning combinations of SO₂ and O₃ injury to vegetation.⁸⁾ MENSER and HEGGESTAD⁷⁾ first noted the synergistic effects of SO₂ and O₃ combination. Thereafter, a number of reports concerning foliar injury response of agricultural crops and native vegetation have been reported.^{2,3,11)} Informations for other pollutant mixtures are limited. TINGEY *et al.*¹⁰⁾ and BENNETT *et al.*¹⁾ have reported the decreased injury threshold and potentiation of symptoms due to combination of SO₂ and NO₂. NO₂ and O₃ occur together in urban areas, however, less is known about the effects of such a combination on plants. Furthermore, there is no known report of plant responses to combination of three pollutants. The following work was therefore undertaken to

determine the effects of SO₂, NO₂ and O₃ singly and in combination of two or three pollutants on net photosynthesis in sunflower plant.

MATERIALS AND METHODS

Plant materials. Sunflower (*Helianthus annuus* L. cv. Russian Mammoth) were grown in a phytotron greenhouse at 25/20°C day/night temperature and 75% RH. Three seeds were sown in 10 cm diameter plastic pots containing 2 : 2 : 1 : 1 (v/v) mixture of peat moss-vermiculite-perlite-fine gravels. About a week after sowing, two of three plants were removed from each pot. Potted plants were watered daily and with Hyponex solution ($\times 1000$) once a week.

Fumigation system. Plant fumigation was performed in an assimilation chamber which was set in a controlled environment room (1.7 $\times$ 2.3 $\times$ 2.0 m high) designed and constructed for studies of the effects of air pollutant combinations on plant. The light source was consisted of twenty-four 400 w stannous halide lamps (Toshiba). The light was filtered through heat absorbing glass filter, which removed radiation above 800 nm. The field air was passed through activated charcoal and catalyst bearing (containing MnOx and CuO) filters to remove ambient pollutants and led into the fumigation room. This filtration system could remove SO₂ and O₃ almost perfectly, but a trace amount of NO₂, below 0.5 pphm, was remained in the room. An air velocity in the environment controlled room was 0.2–0.4 m·sec⁻¹, and a ventilation rate was 30 times·hr⁻¹. SO₂ from a compressed cylinder containing 4000 ppm SO₂ in N₂ was injected through a thermal mass-flow controller into the gas stream. SO₂ concentration was regulated using a controlling system of a pulsed fluorescent SO₂ analyzer (Thermo Electron, Model 43). NO₂ was also supplied from a cylinder containing 500 ppm NO₂ in N₂. NO₂ concentration was regulated by a system similar to that described for SO₂. Chemiluminescent NO-NO₂-NO_x analyzer (Thermo Electron, Model 14) was used to monitor and regulate NO₂ concentration in the room. O₃ was produced by a silent electrical discharge in dry oxygen and regulated by a controlling system of a chemiluminescent O₃ analyzer (Kimoto, Model 806). Recording of the pollutants concentrations inside the room showed that on starting a fumigation, the concentrations reached the fixed level within 5 min. The concentrations of pollutants were regulated within ± 0.5 pphm of the average values.

Photosynthesis measurement. A single attached leaf of 4 to 5 weeks old plants was sealed into a plexiglas assimilation chamber (30 $\times$ 22 $\times$ 1 cm deep). Prior to the fumigation of air pollutants, the leaf was preilluminated for more than an hour to get the steady state of photosynthetic CO₂ uptake at 25°C, 75% RH, and an air flow rate of 10 l·min⁻¹. The quantum flux density inside the assimilation chamber was 50 nE·cm⁻²·sec⁻¹, measured with a quantum flux sensor (Lambda Instruments). The rate of net photosynthesis was determined by measuring the CO₂ concentrations at the inlet and the outlet of the assimilation chamber using an infrared CO₂ analyzer of Shimadzu (URA-2S).

To test if there is a significant difference between two combinations of pollutant mixtures, the F statistic was applied.

RESULTS AND DISCUSSION

Figure 1 shows the inhibition of net photosynthetic rates of sunflower due to

Fig. 1 (A-C) Effects of NO_2 , SO_2 and O_3 separately and combinations of two or three on the rates of net photosynthesis in leaves of sunflower.

The concentration of individual gases is shown in the lower left corner of each subfigure. The rate of net photosynthesis during the exposure to each pollutant is expressed as a ratio of the rate prior to the fumigation. Vertical bars represent standard deviations for each mean of 4 values.

exposures to SO_2 and/or O_3 for 2 hr at various NO_2 concentrations. The rate of net photosynthesis from the time of initiation of exposure (time 0) is expressed as a percent of the rate prior to exposure.

Exposure to the mixture of 0.2 ppm SO_2 /0.2 ppm O_3 for 2 hr reduced the rate of net photosynthesis by 39% of the initial rate. The concentrations of SO_2 and O_3 in the mixture were below or at the photosynthesis inhibition threshold for the individual gases. The exposure to 0.2 ppm SO_2 or 0.2 ppm O_3 separately for 2 hr reduced the rate of net photosynthesis in sunflower by only 2-3%.

The simultaneous application of O_3 with NO_2 inhibited net photosynthetic rates in sunflower to a greater degree than would be expected from summing the effects

due to individual gases. Although this synergistic effect of NO_2 and O_3 mixture should be conceivable, it is curious that the rate of inhibition was not affected by NO_2 concentrations used in the mixture. Mixtures of 1.0 ppm NO_2 /0.2 ppm O_3 and 0.2 ppm NO_2 /0.2 ppm O_3 reduced net photosynthetic rates by 42 and 36% in 2 hr exposures, respectively, but there was no significant difference between these two values on 95% confidence interval.

The effect of NO_2/O_3 mixture on net photosynthesis was quite different from that of SO_2/O_3 mixture. The mixture of SO_2/O_3 caused net photosynthesis to drop rapidly to new equilibrium levels which were then maintained over about 1 hr, but the mixture of NO_2/O_3 caused the net photosynthetic rates to decline steadily. The difference between the inhibition caused by 1 hr fumigation with NO_2/O_3 and that caused by SO_2/O_3 was significant at 95% confidence interval, but there was no detectable difference between the inhibitions caused by 2 hr fumigations of these two mixtures.

The concentration of NO_2 was one variable that influenced the degree of photosynthetic inhibition caused by NO_2/SO_2 mixture. The inhibition caused by 1.0 ppm NO_2 /0.2 ppm SO_2 mixture was significantly greater than the additive effects of the single gases, whereas 0.2 ppm NO_2 /0.2 ppm SO_2 did not enhance the inhibition. WHITE *et al.*¹²⁾ reported that simultaneous fumigations of 0.25 ppm NO_2 /0.2 ppm SO_2 mixture reduced the net photosynthetic rate of alfalfa by 9%. Thus the exposure concentrations of the pollutant mixture to be injurious to photosynthesis in sunflower seemed to be higher than those reported in alfalfa. As the effect of NO_2 on photosynthesis in sunflower was less than that found in alfalfa,⁵⁾ the interspecific difference in response to NO_2/SO_2 mixture may be largely dependent on the difference of sensitivity to NO_2 .

Photosynthetic inhibition caused by $\text{NO}_2/\text{SO}_2/\text{O}_3$ mixtures resembled with those caused by SO_2/O_3 mixtures. Furthermore, the inhibition of net photosynthetic rate due to 2 hr exposure to 1.0 ppm NO_2 /0.2 ppm SO_2 /0.2 ppm O_3 was not significantly different from that caused by 0.2 ppm SO_2 /0.2 ppm O_3 . By contrast, NO_2/O_3 and $\text{NO}_2/\text{SO}_2/\text{O}_3$ mixtures showed different inhibitions of net photosynthesis. Exposure to $\text{NO}_2/\text{SO}_2/\text{O}_3$ mixture rapidly depressed the net photosynthetic rate to equilibrium inhibition levels which was then maintained over 1 hr. The difference between the inhibition caused by NO_2/O_3 and that caused by $\text{NO}_2/\text{SO}_2/\text{O}_3$ were significant at 95% confidence interval in 1 hr fumigation, but no significant difference was detected in 2 hr exposure. The concentration of NO_2 in the mixture of $\text{NO}_2/\text{SO}_2/\text{O}_3$ was not an effective factor which affected the magnitude of photosynthesis inhibition. Two hr exposure to $\text{NO}_2/\text{SO}_2/\text{O}_3$ reduced the rates of net photosynthesis by 43%, whereas the concentration of NO_2 in the mixture was 1.0 or 0.2 ppm. These results and those observed in NO_2/O_3 experiments suggest that NO_2 acts like a catalyst which can not alter the magnitude of photosynthesis inhibition caused by O_3 but only accelerates the inhibitory effect.

Measurement of net photosynthetic rate may be a precise method of assessing possible effects of individual air pollutants and of pollutant mixtures on growth and development of higher plants. It should also be useful in the evaluation of injury induced by pollutant mixtures. However, the effects of pollutant mixture on net photosynthesis have not been investigated sufficiently to develop a complete understanding of synergism or antagonism to be made. Further investigations on this problem are necessary.

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〈和文抄録〉

ヒマワリの光合成速度に対する NO_2 , SO_2 , O_3 の単独および複合影響

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ヒマワリの純光合成速度に対する NO_2 , SO_2 , O_3 の単独処理と混合処理の影響を調べた。使用した汚染質の濃度は、 NO_2 が 1.0 か 0.2ppm, SO_2 と O_3 は 0.2ppm である。

1.0ppm NO_2 , 0.2ppm O_3 , 0.2ppm SO_2 単独処理では光合成速度の低下は観察されなかった。これらの濃度の大气汚染質を2種類もしくは3種類混合して処理すると顕著な光合成速度の減少が見られた。 NO_2 と SO_2 の混合処理では NO_2 の濃度が変わると光合成阻害は変化するが、 NO_2 と O_3 , NO_2 ,

SO₂, O₃ の混合処理の場合には, NO₂ 濃度が 1.0 ppm でも 0.2 ppm でも光合成阻害の割合には差がなかった.

NO₂ と O₃ 混合処理による光合成阻害の傾向は SO₂ と O₃, もしくは NO₂, SO₂, O₃ 混合処理による阻害傾向とはまったく異なっていた. NO₂ と O₃ の混合処理では光合成速度は処理時間に比例して減少したが, SO₂ と O₃, もしくは, NO₂, SO₂, O₃ の混合処理では光合成速度は急激に減少した.